Data Evaluation Record on the Toxicity of Glufosinate-Ammonium to Juvenile Rainbow Trout (*Salmo gairdneri*)

EPA MRID Number 48301105

Data Requirement:	EPA DP Barcode	384955
	OECD Data Point	OECD 204
	EPA MRID	48301105
	EPA Guideline	Non-guideline

Test material: Glufosinate-ammonium **Purity:** 98.9%
Common name Glufosinate-ammonium
Chemical name:
IUPAC: ammonium (2*RS*)-2-amino-4-(methylphosphinato)butyric acid
CAS: 2-amino-4-(hydroxymethylphosphinyl)butanoic acid monoammonium salt
CAS No.: 77182-82-2
Synonyms: Hoe 039866

Primary Reviewer: Christie E. Padova **Signature:** Christie E. Padova
Staff Scientist, CSS-Dynamac Corporation **Date:** 01/18/12

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental Inc. **Date:** 01/30/12

Primary Reviewer: Catherine Aubee **Signature:** C Aubee
Biologist, US EPA/OPP/EFED/ERBIV **Date:** 4 December 2013

EPA PC Code 128850

Date Evaluation Completed: 04-12-2013

CITATION: Fischer, R. 1989. The Effect of Glufosinate-ammonium – Substance, Technical (Identification Code: Hoe 039866 00 ZC99 0001) to *Salmo gairdneri* (Rainbow trout) in a 21-day Prolonged Toxicity Test (Method OECD). Unpublished study performed and sponsored by Hoechst AG, Frankfurt am Main 80, FRG. Laboratory Study No. CE030/89. Study submitted by Bayer CropScience, Stillwell, KS. Study initiated March 17, 1989 and completed April 7, 1989.

EXECUTIVE SUMMARY:

The 21-day (prolonged) toxicity of glufosinate-ammonium (TGAI) to juvenile rainbow trout (*Salmo gairdneri*) was studied under flow-through conditions. Five-month old rainbow trout (*ca.* 5.1 cm and 2.6 g) were exposed to nominal concentrations of 0 (negative control), 5, 10, 50, 100, 500, and 1000 mg/L. Mean-measured concentrations were determined for the control, 5, 50, 100, and 500 mg/L levels only and averaged <LOQ (control), 4.0, 50, 110, and 580 mg ai/L, respectively. The 21-day LC_{50} (with 95% C.I.) was 252.6 (110 to 580) mg ai/L. The 21-day NOAEC was 50 mg ai/L, based on clinical signs of toxicity observed at the ≥110 mg ai/L treatment levels.

Complete mortality occurred at the highest treatment level by Day 1 and at the 580 mg/L level by Day 6. No other mortality occurred during the study. Slow reactions were observed in all fish from the 110 mg/L level on Day 2, and slow reactions, head down or surface swimming, horizontal turns, and/or narcotic conditions were observed from Days 1 through 5 in fish from the 580 mg ai/L exposure level. All surviving fish appeared normal from Day 6 onward. No treatment-related effects were observed on length or weight gain of surviving fish from the ≤110 mg ai/L exposure levels.

This study does not fulfill any current U.S. EPA guideline requirements. This study, however, is scientifically sound, and provides **supplemental** information on the 21-day sub-acute toxicity to rainbow trout (*Salmo gairdneri*).

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Mean of 2.6 g and 5.1 cm (n=70)
Test Type (Flow-through, Static, Static Renewal): Flow-through

Survival and Toxic Signs:
21-Day LC_{50}: 252.6 mg ai/L 95% C.I.: 110 to 580 mg ai/L
Slope: N/A
NOAEC: 50 mg ai/L
LOAEC: 110 mg ai/L (clinical signs of toxicity)

Growth:
NOAEC: 110 mg ai/L
LOAEC: ≥110 mg ai/L (not determined at higher treatment levels due to mortality)

I. MATERIALS AND METHODS

GUIDELINE(S) FOLLOWED: The study protocol followed OECD Guideline No. 204 (1984).

This study does not follow any current U.S. EPA OCSPP Guideline. There were no notable deviations or deficiencies from OECD 204 observed. It was reported that the study was extended (from 14) to 21 days due to a request of the German Registration Authorities.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material Glufosinate-ammonium (technical grade)

Description: White powder

Lot No./Batch No.: Identification Code Hoe 039866 00 ZC99 0001

Purity: 98.8%

Stability of compound under test conditions: Stable, as indicated by regular analysis of test water (refer to Appendix 2); calculated CVs were ≤9.0% for all treatment levels.

Storage conditions of test chemicals: Not reported

Physicochemical properties of glufosinate-ammonium.

Parameter	Values	Comments
Water solubility at 20°C	1370 g/L	At 22°C and pH 5
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species: Rainbow trout (*Salmo gairdneri*)

Age at test initiation: *Ca.* 5 months old

Size: Mean weight – 2.6 g (n=70; SD not reported)

Mean length – 5.1 cm (n=70; SD not reported)

Source: Eggs were obtained from Steinhardt trout hatchery, Hettingen, FRG

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: None performed. It was reported that concentrations selected for use in the definitive test were based on previously-conducted studies (not otherwise specified).

c. Definitive study

Table 1: Experimental Parameters

Parameter	Details	Remarks
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	7 days Same as test Six times per week with standard trout food (Rheinkrone, Wesel, FRG), Tetra-Min, frozen fly larvae, frozen waterfleas, and freshly-minced beef liver. 0.2% in the stock culture over the 21-day period immediately preceding the definitive study	Eggs were hatched and maintained at the laboratory under flow-through conditions in fiber glass tanks. Upon receipt, eggs were prophylactically-treated with 8 mg Actomar K30 per liter water for 15 minutes.
Number of organisms in each treatment at test initiation	10 fish per level	Fulfills OECD requirements.
Biomass loading rate	0.051 g/L/day (flow-through) 0.52 g/L (instantaneous)	Calculated at study initiation; fulfills OECD requirements.

Parameter	Details	Remarks
Concentration of test material nominal: mean measured:	0 (negative control), 5, 10, 50, 100, and 500 mg/L 0 (control), 4.0, --, 50, 110, and 580 mg ai/L, respectively.	For concentration verification, samples were collected from the 0 (control), 5, 50, 100, and 500 mg/L levels on Days 4, 11, and 18. Water samples were analyzed by direct-injection HPLC with UV (195 nm) detection. Mean-measured concentrations were reviewer-calculated (see copy of Excel worksheet in Appendix 2). Concentrations were satisfactorily maintained at ≥80% of nominal concentrations. In addition, minimum analytical variation was observed, with reviewer-calculated coefficients of variation (CVs) of ≤9.0% (Appendix 2).
Solvent (type, percentage, if used)	N/A	
Number of replicates control: solvent control: treated ones:	 1 N/A 1/level	The use of multiple replicates is not delineated in OECD 204 guidance.
Test condition static renewal/flow-through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 Flow-through Intermittent-introduction diluter 350 ± mL/minute ± 10% N/A	Flow rate of the diluter was verified daily. The turnover rate was equivalent to *ca.* 10 volume additions/day (reviewer-calculated) using the following formula: (350 mL/min) x (1 L/1000 mL) x (1 volume addition/50 L) x (60 min/ hr) x (24 hr/day).

Parameter	Details	Remarks
Aeration, if any	No supplemental aeration was provided.	
Duration of the test	21 days	
Test vessel type/material: (glass/stainless steel) size: fill volume:	 Stainless steel 40 x 30 x 50 cm 50 L (33.5 to 33.7 cm depth)	
Source of dilution water	Dilution water was tap water that was sand-filtered, charcoal-filtered. Dilution water was aerated to oxygen saturation and brought to 14 ± 1°C in the storage tank.	
Water parameters hardness: pH: dissolved oxygen: temperature: photoperiod: other measurements: interval of water quality measurements:	 317 to 340 mg/L as $CaCO_3$ 7.8 to 8.1 (8.0 ± 0.11) 9.6 to 11.6 mg/L (10.5 ± 0.37 mg/L) 13.3 to 14.5°C (13.7 ± 0.27°C) 16-hours light/8- hour dark Conductivity 647 to 1290µmhos/cm Temperature, pH, and DO were measured daily in each tank. Temperature was also continuously recorded in each tank. Total hardness, total alkalinity, sodium, potassium, and nitrite concentrations were determined in the dilution water at study initiation and termination.	Analysis of the dilution water at the beginning and end of the test yielded the following results: total hardness 317-340 mg/L as $CaCO_3$, total alkalinity 249-260 mg/L as $CaCO_3$, sodium 13-17 mg/L, potassium 2.3-3.3 mg/L, and nitrite <0.05 mg/L.

Parameter	Details	Remarks
Feeding type/source of feed: amount given: frequency of feeding:	 Commercial, pelleted dry fish food 0.50 to 0.58 g dry food per tank per day Once daily	
Recovery of chemical: Frequency of measurement: LOD: LOQ:	Mean of 101.5% of nominal Days 4, 11, and 18 Not reported Not reported	Based on mean-measured concentrations.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	- Mortality - Sub-lethal effects - Body length of surviving fish - Wet weight of surviving fish	
Observation intervals:	Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Cumulative mortality was 0% in the control group and the nominal 5, 10, 50, and 100 mg/L exposure levels, and 100% in the nominal 500 and 1000 mg/L exposure levels. Complete mortality occurred at the highest treatment level by Day 1 and at the 500 mg/L level by Day 6. The 21-Day LC_{50} was 224 mg/L, with 95% confidence levels of 100 to 500 mg/L using nominal concentrations. The NOAEC for mortality was 100 mg/L.

B. SUB-LETHAL TOXICITY AND OTHER EFFECTS:

Clinical signs of toxicity were predominantly observed in fish from the 500 mg/L level. Effects were observed from Days 1 through 5 and included slow reactions, head down or surface swimming, horizontal turns, and/or narcotic conditions. Slow reactions were also observed in all fish from the 100 mg/L level on Day 2; however, the study author reported that since this observation only occurred once, it was concluded to be not exposure-related (see Reviewer's Comments section). The reported NOAEC for clinical signs of toxicity was 100 mg/L.

At study termination, lengths and weights were determined for all surviving fish. For all levels (including the control), lengths ranged from 5.92 to 6.39 cm and weights ranged from 3.65 to 4.32 g, with no statistically-significant differences indicated for either parameter compared to the control. The NOAEC for both growth parameters was 100 mg/L.

Table 3: Mortality and Sub-lethal Effects (Effect Code/Number Affected) of Glufosinate-ammonium on Juvenile Rainbow Trout.

Treatment Mean-measured (and nominal) conc., mg/L[(a)]	No. offish at start of study	Observation period											
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
		No. dead	Other effects	No. dead	Other effects	No. dead	Other effects	No. dead	Other effects	No. dead	Other effects	No. dead	Other effects
Control (dilution water only)	10	0	N	0	N	0	N	0	N	0	N	0	N
4.0 (5.0)	10	0	N	0	N	0	N	0	N	0	N	0	N
--- (10)	10	0	N	0	N	0	N	0	N	0	N	0	N
50 (50)	10	0	N	0	N	0	N	0	N	0	N	0	N
106 (100)	10	0	N	0	S1/10	0	N	0	N	0	N	0	N
580 (500)	10	7	S1/3 S2/3	7	S1/3 S3/2 S4/1	7	S1/1 S2/2 S4/2 S5/2	7	S1/3 S4/3	7	S5/3	10	N/A
--- (1000)	10	10	N/A	10	N/A	10	N/A	10	N/A	10	N/A	10	N/A
NOAEC, mg/L		100											
LC_{50} (95% C.I.), mg/L		224 (100 to 500)											

[(a)] Concentration verification was not performed at the nominal 10 and 1000 mg/L treatment levels.
N = normal, S1 = slow reactions, S2 =head down swimming, S3 = surface swimming, S4 = horizontal turns, S5 = narcotic condition, N/A = not applicable.

Table 4: Effect of Glufosinate-ammonium on Growth of Juvenile Rainbow Trout.

Treatment Mean-measured (and nominal) conc., mg/L[(a)]	Mean Length (cm ± SD)	Mean Weight (g ± SD)
Control (dilution water only)	6.18 ± 0.623	4.08 ± 1.090
4.0 (5.0)	6.16 ± 0.578	3.88 ± 1.112
--- (10)	6.39 ± 0.552	4.32 ± 1.015
50 (50)	6.15 ± 0.314	3.82 ± 0.803
106 (100)	5.92 ± 0.771	3.65 ± 1.139
580 (500)	N/A	N/A
--- (1000)	N/A	N/A
NOAEC (mg/L)	100	
LOAEC (mg/L)	>100	

[(a)] Concentration verification was not performed at the nominal 10 and 1000 mg/L treatment levels.

C. REPORTED STATISTICS:

The 21-Day LC_{50} values with associated 95% confidence intervals were calculated using a computerized LC_{50} program developed by Stephan *et al.* (1978). The program calculates the LC_{50} and its 95% confidence intervals using the bionomial, moving average, and probit tests, and the method selected for reporting was that which gave the narrowest confidence intervals.

Growth data (untransformed) were assessed by one-way analysis of variance (ANOVA), General Linear Models, and Duncan's Multiple Range Test Procedures. Analyses were performed using SAS (1979) statistical software. All results were reported in terms of nominal concentrations.

21-Day LC_{50}: 224 mg/L 95% C.I.: 100 to 500 mg/L
NOAEC: 100 mg/L (based on mortality and clinical signs of toxicity)
LOAEC: 0.046 mg/L (based on mortality and clinical signs of toxicity)

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer verified the results for survival, length and wet weight. The reviewer calculated the % increase in length and weight for each fish using the Day 0 and 28 data. These data were confirmed to be normally distributed using the Shapiro-Wilk's test and the variances were homogeneous, as confirmed using Levene's test. The NOAEC and LOAEC were determined using ANOVA, followed by Dunnett's test. These analyses were conducted using Toxstat 3.5 statistical software. The reviewer additionally confirmed the 21-day LC_{50} using the binomial method via Toxanal 2009 software; neither the Probit nor moving average method were appropriate fits for this data set.

Toxic Signs:
21-Day LC_{50}: 252.6 mg ai/L 95% C.I.: 110 to 580 mg ai/L
Slope: N/A
NOAEC: 50 mg ai/L
LOAEC: 110 mg ai/L

Growth:
NOAEC: 110 mg ai/L
LOAEC: ≥110 mg ai/L

E. STUDY DEFICIENCIES:

This study is scientifically sound; no notable deviations from OECD 204 Guidance were observed.

F. REVIEWER'S COMMENTS:

The reviewer's statistical conclusions regarding mortality and growthwere similar to the study author's, but conclusions were expressed based on the mean-measured concentrations. Slow reactions were observed in all fish from the 100 mg/L exposure level on Day 2. The study author reported that since this observation occurred only once, it was regarded to be not treatment-related. The reviewer does not support this conclusion as slow reactions were not observed in control fish, but were observed in up to three surviving fish exposed at 500 mg/L on Days 1 through 4 before subsiding. In addition, there was a 5-fold difference between the 100 and 500 mg/L treatment levels, compared to a 2-fold difference between the 50 and 100 mg/L treatment levels. Thus, a treatment-related effect cannot be excluded and the NOAEC for the study is determined to be 50 mg/L based on toxic signs.

All results were reported in terms of mean-measured concentrations in the Executive Summary and Conclusions sections of the DER.

Water samples were analyzed by direct-injection HPLC with UV (195 nm) detection. Although not delineated in OECD 204 Guidance, the analytical LOD and/or LOQ should have been reported, as well as any results for procedural recovery, matrix blank, etc. samples.

Experimental test dates were March 17 to April 7, 1989.

G. CONCLUSIONS:

This study is scientifically sound and provides **supplemental** information on the 21-day toxicity to juvenile rainbow trout (*Salmo gairdneri*). The 21-day LC_{50} was 252.6 mg ai/L, with 95% C.I. of 110 to 580 mg ai/L. Treatment-related mortality was observed at the ≥580 mg ai/L exposure levels, and treatment-related clinical signs of toxicity were observed at the ≥110 mg ai/L exposure levels; thus, the NOAEC and LOAEC were 50 and 110 mg ai/L, respectively, based on transient but concentration-responsive clinical signs of toxicity which coincided with mortality at higher treatment levels. No treatment-related affect on terminal growth (length and wet weight) was observed in surviving fish from the ≤110 mg ai/L levels.

Survival and Toxic Signs:
21-Day LC_{50}: 252.6 mg ai/L 95% C.I.: 110 to 580 mg ai/L
Slope: N/A
NOAEC: 50 mg ai/L
LOAEC: 110 mg ai/L

Growth:
NOAEC: 110 mg ai/L
LOAEC: ≥110 mg ai/L

III. REFERENCES:

Hoechst AG. 1983. Hoe 039866, Solubility in water. Report (B)11/83, Document No. A 25476. Hoechst AG, D-6230 Frankfurt am Main 80, 1983-03-10.

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SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute Inc., Cary, NC 27511.

Stephen, C.E., *et al.* 1978. A Computer Program for Calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota, Pre-publication manuscript, August 1978.

Laird, C.E. 1986. U.S. Environmental Protection Agency. Pesticide Assessment Guidelines, subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 72-1 Acute Toxicity Freshwater Fish, Addendum on Data Reporting.

APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Title:  Length % gain
File:             11051             Transform:              NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
--------------------------------------------------------------------------------

     D = 9413.2570
     W =   0.9692

     Critical W  = 0.9300 (alpha = 0.01 , N = 50)
              W  = 0.9470 (alpha = 0.05 , N = 50)

--------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.



Title:  Length % gain
File:             11051             Transform:              NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             4            471.6248         117.9062       1.5764

   Within (Error)     45           3365.8230          74.7961
--------------------------------------------------------------------------------
   Total              49           3837.4478
--------------------------------------------------------------------------------
                                                        (p-value = 0.1970)

   Critical F  = 3.7674  (alpha = 0.01, df = 4,45)
               = 2.5787  (alpha = 0.05, df = 4,45)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)

Title:  Length % gain
File:             11051             Transform:              NO TRANSFORMATION

                               ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             4             83.8152          20.9538       0.1002

   Within (Error)     45           9413.2570         209.1835
--------------------------------------------------------------------------------
   Total              49           9497.0722
--------------------------------------------------------------------------------
                                                        (p-value = 0.9818)
```

```
     Critical F  = 3.7674  (alpha = 0.01, df = 4,45)
                 = 2.5787  (alpha = 0.05, df = 4,45)

     Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.05)


  Title:  Length % gain
  File:             1105l             Transform:             NO TRANSFORMATION

        Dunnett's Test   -   TABLE 1 OF 2             Ho:Control<Treatment
  ------------------------------------------------------------------------------
                                TRANSFORMED    MEAN CALCULATED IN          SIG
  GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT
0.05
  ----- --------------------    -----------    ------------------  ------  ---
    1                Control     19.9300            19.9300
    2                 5 mg/L     18.0200            18.0200        0.2953
    3                10 mg/L     20.4800            20.4800       -0.0850
    4                50 mg/L     21.8000            21.8000       -0.2891
    5               100 mg/L     18.9400            18.9400        0.1531
  ------------------------------------------------------------------------------
  Dunnett critical value = 2.2300   (1 Tailed, alpha = 0.05, df [used] = 4,40)
                                                              (Actual df = 4,45)


  Title:  Length % gain
  File:             1105l             Transform:             NO TRANSFORMATION

        Dunnett's Test   -   TABLE 2 OF 2             Ho:Control<Treatment
  ------------------------------------------------------------------------------
                              NUM OF     MIN SIG DIFF     % OF     DIFFERENCE
  GROUP    IDENTIFICATION     REPS     (IN ORIG. UNITS)  CONTROL  FROM CONTROL
  ----- --------------------  -------  ----------------  -------  ------------
    1                Control    10
    2                 5 mg/L    10           14.4239       72.4        1.9100
    3                10 mg/L    10           14.4239       72.4       -0.5500
    4                50 mg/L    10           14.4239       72.4       -1.8700
    5               100 mg/L    10           14.4239       72.4        0.9900
  ------------------------------------------------------------------------------


  Title:  Length % gain
  File:             1105l             Transform:             NO TRANSFORMATION

         William's Test  - TABLE 1 OF 2        Ho: Control<Treatment
  ------------------------------------------------------------------------------
                                     ORIGINAL      TRANSFORMED    ISOTONIZED

  GROUP     IDENTIFICATION       N      MEAN           MEAN           MEAN
  ------  --------------------  ---  -----------    -----------    -----------
     1                 Control  10     19.9300        19.9300        20.0575
     2                  5 mg/L  10     18.0200        18.0200        20.0575
```

3	10 mg/L	10	20.4800	20.4800	20.0575
4	50 mg/L	10	21.8000	21.8000	20.0575
5	100 mg/L	10	18.9400	18.9400	18.9400

Title: Length % gain
File: 1105l Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Control	19.9300				
5 mg/L	20.0575	-0.0197		1.6800	k= 1, v=40
10 mg/L	20.0575	-0.0197		1.7600	k= 2, v=40
50 mg/L	20.0575	-0.0197		1.7900	k= 3, v=40
100 mg/L	18.9400	0.1531		1.8000	k= 4, v=40

s = 14.4632

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Title: Weight % gain
File: 1105w Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 128064.1910
W = 0.9481

Critical W = 0.9300 (alpha = 0.01 , N = 50)
W = 0.9470 (alpha = 0.05 , N = 50)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Weight % gain
File: 1105w Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	4	1655.8628	413.9657	0.3217
Within (Error)	45	57906.6000	1286.8133	

```
  ------------------------------------------------------------------------------
     Total              49          59562.4628
  ------------------------------------------------------------------------------
                                                              (p-value = 0.8620)

     Critical F  = 3.7674  (alpha = 0.01, df = 4,45)
                 = 2.5787  (alpha = 0.05, df = 4,45)

     Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)

  Title:  Weight % gain
  File:             1105w             Transform:              NO TRANSFORMATION

                                ANOVA Table
  ------------------------------------------------------------------------------

     SOURCE             DF               SS                MS           F
  ------------------------------------------------------------------------------
     Between             4            131.9540          32.9885       0.0116

     Within (Error)     45         128064.1910        2845.8709
  ------------------------------------------------------------------------------
     Total              49         128196.1450
  ------------------------------------------------------------------------------
                                                              (p-value = 0.9997)

     Critical F  = 3.7674  (alpha = 0.01, df = 4,45)
                 = 2.5787  (alpha = 0.05, df = 4,45)

     Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.05)



  Title:  Weight % gain
  File:             1105w             Transform:              NO TRANSFORMATION

        Dunnett's Test   -   TABLE 1 OF 2             Ho:Control<Treatment
  ------------------------------------------------------------------------------
                                TRANSFORMED    MEAN CALCULATED IN           SIG
  GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT
0.05
  ----- --------------------    -----------    ------------------  ------  ---
    1               Control     55.5100            55.5100
    2                5 mg/L     54.4600            54.4600         0.0440
    3               10 mg/L     56.8700            56.8700        -0.0570
    4               50 mg/L     57.2800            57.2800        -0.0742
    5              100 mg/L     52.8300            52.8300         0.1123
  ------------------------------------------------------------------------------
  Dunnett critical value = 2.2300   (1 Tailed, alpha = 0.05, df [used] = 4,40)
                                                              (Actual df = 4,45)



  Title:  Weight % gain
  File:             1105w             Transform:              NO TRANSFORMATION

        Dunnett's Test   -   TABLE 2 OF 2             Ho:Control<Treatment
```

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Control	10			
2	5 mg/L	10	53.2019	95.8	1.0500
3	10 mg/L	10	53.2019	95.8	-1.3600
4	50 mg/L	10	53.2019	95.8	-1.7700
5	100 mg/L	10	53.2019	95.8	2.6800

Title: Weight % gain
File: 1105w Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	10	55.5100	55.5100	56.0300
2	5 mg/L	10	54.4600	54.4600	56.0300
3	10 mg/L	10	56.8700	56.8700	56.0300
4	50 mg/L	10	57.2800	57.2800	56.0300
5	100 mg/L	10	52.8300	52.8300	52.8300

Title: Weight % gain
File: 1105w Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Control	55.5100				
5 mg/L	56.0300	-0.0218		1.6800	k= 1, v=40
10 mg/L	56.0300	-0.0218		1.7600	k= 2, v=40
50 mg/L	56.0300	-0.0218		1.7900	k= 3, v=40
100 mg/L	52.8300	0.1123		1.8000	k= 4, v=40

s = 53.3467

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

APPENDIX 2: COPY OF REVIEWER'S COEFFICIENT OF VARIATION (CV) CALCULATIONS:

Nominal Concentration (mg/L)	Time (Day)	Measured Concentration (mg ai/L)	
5	4	3.7	
	11	3.9	
	18	4.4	
		Mean	**4.0**
		Standard Dev	0.3605551
		CV (%)	9.0
50	4	49.6	
	11	48.6	
	18	52.7	
		Mean	**50.3**
		Standard Dev	2.1377558
		CV (%)	4.3
100	4		
	11	103.2	
	18	109.7	
		Mean	**106.5**
		Standard Dev	4.5961941
		CV (%)	4.3
500	4	581.9	
	11		
	18		
		Mean	**581.9**
		Standard Dev	#DIV/0!
		CV (%)	#DIV/0!